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Pharmacokinetics of chloroquine in Indian tribal and non-tribal healthy volunteers and patients with *Plasmodium falciparum* malaria

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The pharmacokinetics of chloroquine was studied in Indian tribal and non-tribal healthy volunteers and patients infected with *Plasmodium falciparum* malaria, after a single dose of 600 mg chloroquine. Mean area under the curve (AUC), half-life ($T_{1/2}$) and peak concentration (C_{max}) in tribal *P. falciparum* patients were 18.79 ± 5.82 μg h ml^{-1}, 115.94 ± 57.71 h and 435 ± 135.17 ng ml^{-1} respectively, while in non-tribal *P. falciparum* patients they were 17.00 ± 5.60 μg h ml^{-1}, 76.15 ± 8.00 h and 454 ± 193 ng ml^{-1} respectively. Pharmacokinetic parameters did not appreciably differ between tribal and non-tribal groups of subjects in healthy volunteers or in *P. falciparum* patients. However, the time to reach maximum concentration (T_{max}) was 8 h in tribal subjects and 4 h in non-tribal subjects. Mean ratio of AUC of chloroquine to desethylchloroquine in tribal *P. falciparum* patients was higher (4.26 ± 1.34) than non-tribal subjects (3.41 ± 0.66), suggesting reduced chloroquine metabolism in tribal subjects. However, the difference was statistically insignificant ($t = 1.35$, $P < 0.5$). Delayed T_{max} and impaired chloroquine metabolism may be associated with general health problems such as malnutrition, anaemia and parasitic infestations of the tribal population in India.

CHLOROQUINE has long been the drug of choice for the treatment and prevention of malaria in India. The pharmacokinetics of chloroquine has been studied in different ethnic groups and conditions, e.g. Caucasians[1], Swedish[2] and Thais[3]. However, the pharmacokinetics does not differ substantially among various ethnic groups. Walker and coworkers[4] have compared disposition of chloroquine between kwashiorkor and normal children, and found decreased chloroquine absorption in patients with kwashiorkor. Little is known about the pharmacokinetics of chloroquine in malnourished patients. Tulpule and Krishnaswamy[5] found faster clearance of chloroquine in undernourished subjects. Tribals contribute 7.95% of the total population in India and 23.73% of them live in Madhya Pradesh (MP)[6,7]. High prevalence of malnutrition (60%), anaemia (40%; Hb < 7 g%) and parasitic infestations (75%)[7] was observed in the tribal population from MP. We now report plasma chloroquine pharmacokinetics in tribal and non-tribal healthy volunteers and patients with *Plasmodium falciparum* malaria, after a single oral dose of 600 mg chloroquine.

Studies on tribal subjects were carried out at the Bijadandi Primary Health Centre (PHC), District Mandla, MP, India under the supervision of PHC doctors, while the studies on non-tribal subjects were performed at the main hospital of the Indian Oil Corporation (IOC), Mathura, India.

A total of 12 subjects were selected from the tribal forest area. Six of these (4 males and 2 females) were healthy volunteers, while another six (4 males and 2 females) were patients with *P. falciparum* infection with a mean parasite density of 1720 mm^3 (range 1200–2320). Five *P. falciparum*-infected non-tribal patients (4 males and 1 female) with mean parasite density of 2688 mm^3 (range 800–7680) and five male healthy volunteers were also selected for comparison. Patients and volunteers were adults and selected randomly depending on their willingness to participate in the study, and their numbers were based on earlier reports[2,4] along with the feasibility of blood sampling in tribal areas.

All tribal subjects were malnourished, having an anthropometric index[5] below 0.18 and their mean haemoglobin levels were 8.5 g%. Mean anthropometric index and haemoglobin levels of non-tribal population were > 0.20 and > 11.0 g% respectively. No investigation for parasitic infestation was performed on any tribal subject.

The study was confined to those subjects who did not have any other disease or abnormality along with

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malaria, and had not taken chloroquine or any other drug for fever previously. Chloroquine absorption was checked by urine test[8]. The study protocol was approved by the Institutional Review Committee, Malaria Research Centre (ICMR), New Delhi. The mean age of healthy tribal subjects and *P. falciparum* patients was 29 and 30 years, while their body weights were 45 and 46 kg respectively. Mean age and body weight of non-tribal *P. falciparum* patients were 32 years and 56 kg, while those of healthy volunteers were 34 years and 58 kg respectively. Each subject was given a single oral dose of 600 mg chloroquine base (4 tablets of chloroquine phosphate supplied by the National Eradication Programme of India) and admitted to the study in the PHC.

Venous blood samples (2.0 ml) were drawn from tribal subjects into heparinized glass tubes from an indwelling catheter in an antecubital vein before, and at 0.5, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0, 10.0, 24.0, 48.0, 96.0 and 168 h after administration of chloroquine. All blood samples were transported in a specially designed ice-packed container within 30 min of each collection to a biochemical laboratory of the Post-Graduate Medical College, Jabalpur in the case of tribal subjects. All tubes were kept in a horizontal position and centrifuged at 1500 *g* for 15 min. Plasma samples were collected after removing the buffy coat and stored at – 20°C until analysis. Blood samples of non-tribal cases were centrifuged immediately after collection at the pathology laboratory of IOC, Mathura.

A high-performance liquid chromatographic method described by Alván *et al.*[9] was used to determine chloroquine and its metabolite desethylchloroquine concentrations in the plasma. To separate chloroquine and its metabolites from endogenous components, acetonitrile–methanol–diethylamine, mixed in the ratio of 60 : 10 : 0.5, v/v, was used as mobile phase at a flow rate of 1.0 ml min^{-1} and delivered to a μPorasil (3.9 mm × 300 mm) normal phase column. Chloroquine and desethylchloroquine were detected by a fluorescence spectrophotometer at an excitation wavelength of 350 nm and an emission wavelength of 390 nm. The retention times of chloroquine and desethylchloroquine were 8.8 and 12.8 min respectively. The detection limits for chloroquine and desethylchloroquine were 1 ng. Mean within-day and day-to-day coefficients of variation (CV) in the plasma for chloroquine averaged 2.3 and 2.76% respectively, while for desethylchloroquine they were 2.83 and 3.36% respectively.

C_{max} and T_{max} were noted directly from plasma chloroquine concentrations at different intervals after dosing. Plasma concentration versus time profiles were analysed by nonlinear least squares regression (TOPFIT® Schering, Germany) and all pharmacological parameters such as elimination half-life ($T_{1/2}$) and clearance time (Cl) were calculated using data point up to 168 h, while area under the curve (AUC) was calculated 0–168 h and at 0–∞ using TOPFIT® Software (Schering, Germany). Student's *t* test was used for statistical comparison of the two groups of data.

Tribal subjects have significantly lower body weight than non-tribals ($P < 0.05$). Mean plasma chloroquine and desethylchloroquine concentrations in *P. falciparum*, healthy tribal and non-tribal subjects at different times after a single oral dose of 600 mg chloroquine are given in Table 1. Chloroquine C_{max} in *P. falciparum* tribal and non-tribal patients ranged from 300 to 680 ng ml^{-1} (435 ± 135.17) and 250 to 750 ng ml^{-1} (454 ± 193) respectively, while C_{max} of tribal and non-tribal healthy volunteers ranged from 166 to 600 ng ml^{-1} (280.16 ± 162.30) and 200 to 620 ng ml^{-1} (360 ± 159) respectively.

Table 1. Mean plasma chloroquine (CQ) and desethylchloroquine (DCQ) concentration in tribal and non-tribal healthy volunteers and *P. falciparum* patients

	Concentration (ng ml^{-1})[#]							
	Healthy				*P. falciparum*			
	CQ		DCQ		CQ		DCQ	
Time (h)	Tribal	Non-tribal	Tribal	Non-tribal	Tribal	Non-tribal	Tribal	Non-tribal
0.5	30 ± 25	57 ± 12	4 ± 0.4	20 ± 8	34 ± 17	67 ± 10	11 ± 7	25 ± 12
1.5	100 ± 19	88 ± 19	14 ± 5	29 ± 7	50 ± 23	112 ± 26	19 ± 9	40 ± 16
2.0	120 ± 50	156 ± 34	36 ± 11	50 ± 10	71 ± 16	174 ± 50	22 ± 13	58 ± 24
3.0	150 ± 26	240 ± 71	48 ± 12	86 ± 24	91 ± 28	274 ± 110	26 ± 8	84 ± 27
4.0	210 ± 110	360 ± 159	53 ± 14	134 ± 47	176 ± 56	454 ± 193	39 ± 20	146 ± 34
5.0	230 ± 134	310 ± 143	60 ± 18	106 ± 24	220 ± 65	352 ± 147	55 ± 22	112 ± 19
6.0	250 ± 166	246 ± 105	62 ± 16	84 ± 23	285 ± 97	278 ± 108	79 ± 21	88 ± 16
8.0	280 ± 162	194 ± 57	67 ± 18	63 ± 26	435 ± 135	240 ± 84	95 ± 27	68 ± 19
10.0	230 ± 111	156 ± 45	57 ± 18	49 ± 22	283 ± 103	198 ± 70	68 ± 32	54 ± 28
24.0	170 ± 69	128 ± 34	46 ± 15	39 ± 21	179 ± 63	158 ± 59	46 ± 22	41 ± 14
48.0	110 ± 53	100 ± 38	36 ± 8	33 ± 20	137 ± 49	114 ± 33	40 ± 24	34 ± 13
96.0	60 ± 5	66 ± 22	16 ± 6	25 ± 10	85 ± 28	70 ± 21	17 ± 16	24 ± 10
168.0	20 ± 6	34 ± 11	8 ± 1	14 ± 4	32 ± 10	52 ± 23	9 ± 6	16 ± 7

[#]Values are in mean ± SD.

Table 2. Chloroquine pharmacokinetic parameters in tribal and non-tribal healthy and *P. falciparum* subjects

Subject	Pharmacokinetic parameter					
	C_{max} (ng ml^{-1})*	T_{max} (h)	$T_{1/2}$ (h)	CL (ml min^{-1})	AUC (0–168 h) (µg h ml^{-1})	AUC (0–∞) (µg h ml^{-1})
Healthy						
Tribal	166–600 (280.16 ± 162.30)	8	67.02 (± 16.04)	619.67 (± 146.44)	15.14 (± 5.14)	17.09 (± 4.97)
Non-tribal	200–620 (360 ± 159)	4	70.54 (± 10.02)	601.60 (± 170.34)	14.36 (± 4.55)	17.86 (± 5.69)
P. falciparum						
Tribal	300–680 (435 ± 135.16)	8	115.94 (± 57.71)	456.33 (± 178.33)	18.79 (± 5.82)	24.62 (± 8.52)
Non-tribal	250–750 (454 ± 193)	4	76.15 (± 8)	494.60 (± 195.03)	17.00 (± 5.60)	22.70 (± 8.48)

Range of values.
*Values in parentheses indicate mean ± SD.

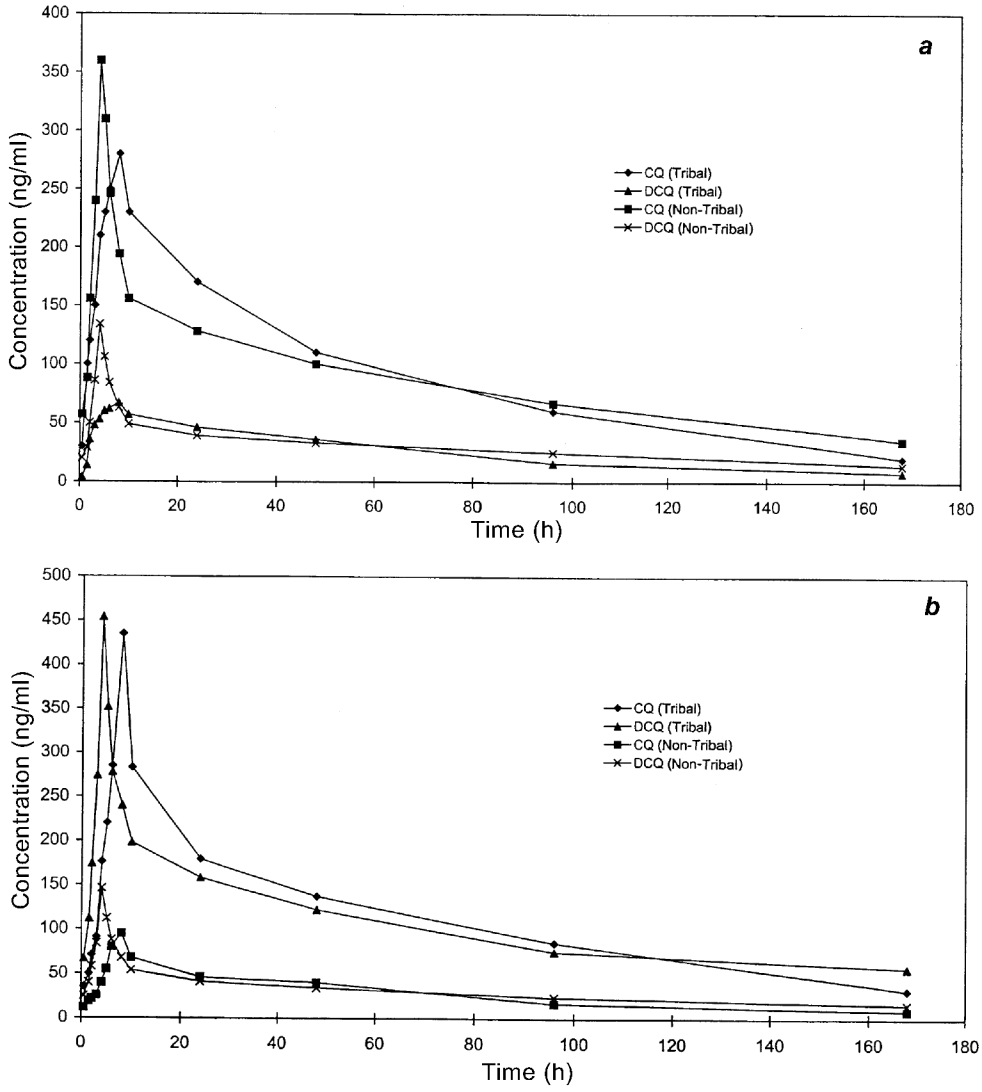

Figure 1. Mean plasma chloroquine (CQ) and desethylchloroquine (DCQ) concentration vs time curve following a single dose of 600 mg chloroquine base to ***a***, Healthy tribal (n = 6) and non-tribal (n = 5) volunteers; and ***b***, *P. falciparum* tribal (n = 6) and non-tribal (n = 5) cases.

C_{max} of desethylchloroquine in *P. falciparum* tribal and non-tribal patients was 95 and 146 ng ml^{-1} respectively. Time to reach peak concentration (T_{max}) in tribal and non-tribal subjects was 8 and 4 h respectively. Plasma chloroquine pharmacokinetic parameters in tribal and non-tribal subjects are given in Table 2. Mean terminal half-life ($T_{1/2}$) in *P. falciparum* tribal and non-tribal patients was 115.94 ± 57.71 and 76.15 ± 8 h respectively, while in healthy volunteers the corresponding values were 67.02 ± 16.04 and 70.54 ± 10.02 h respectively. Mean clearance in *P. falciparum* tribal and non-tribal subjects was 456.33 ± 178.38 and 494.60 ± 195.03 ml min^{-1} respectively. Figure 1 *a* and *b* show the plasma chloroquine and desetheylchloroquine concentrations versus time profile of healthy and tribal and non-tribal *P. falciparum* subjects. Mean AUC (0–168 h) values for *P. falciparum* tribal and non-tribal cases were 18.79 ± 5.82 and 17.00 ± 5.60 μg h ml^{-1} respectively, while for healthy tribal and non-tribal cases they were 15.14 ± 5.14 and 14.36 ± 4.55 μg h ml^{-1} respectively. Mean AUC (0–∞) for tribal and non-tribal *P. falciparum* patients as calculated by TOPFIT® program was 24.62 and 22.70 μg h ml^{-1} respectively (Table 2).

Chloroquine plasma pharmacokinetics in *P. falciparum* and healthy tribal and non-tribal subjects did not differ appreciably. The difference between AUCs varied from 11 to 25% for various groups. AUCs were similar in healthy and *P. falciparum* malaria subjects. Edwards *et al.*[10] found that acute *P. vivax* malaria did not alter plasma pharmacokinetics of chloroquine. The terminal half-life in healthy volunteers and *P. falciparum* patients was similar to earlier findings[11,12]. However, a very long half-life was also recorded by some groups[2,3]. Plasma concentration profiles in *P. falciparum* patients were generally in excess of those in healthy volunteers. This may be due to increased chloroquine uptake by malaria parasites[12]. Chloroquine concentration in all cases was above the therapeutic concentration of 16 μg l^{-1} throughout the study period of 168 h.

The major differences observed in chloroquine pharmacokinetic studies of tribal and non-tribal subjects and with reference to an earlier report[13] relate to the time to reach peak plasma concentration and the ratio of chloroquine to desethylchloroquine. Time to reach peak concentration (T_{max}) in tribal subjects was 8 h, while in non-tribal ones it was 4 h. It should be noted that T_{max} in tribal subjects is in general longer than that previously reported[13,14]. Ette *et al.*[14] have reported that individuals with longer T_{max} values had lower C_{max}, which was due to slow absorption of the drug. A similar trend was seen in our study of tribal cases where longer T_{max} resulted in lower C_{max} compared to non-tribal subjects.

The ratio of chloroquine to desethylchloroquine in tribal subjects was higher than non-tribal ones in the present study, and is also more compared to previous reports[13,15]. This is further evident by comparison of the ratio of AUC of chloroquine to desethylchloroquine in *P. falciparum* infected tribal and non-tribal populations, which was 4.26 and 3.41 respectively. This indicates that chloroquine metabolism in tribal subjects is reduced compared to non-tribal ones; however, the difference was not statistically significant ($t = 1.35$, $P < 0.5$). This difference may reflect the general health problems of tribal populations in India, such as malnutrition, anaemia and parasitic infestation.

Although chloroquine concentration exceeded the targeted therapeutic concentration of 16 μg l^{-1} after 0.5 h of dosing, and remained high in all groups of subjects under the study period[16], however, delayed T_{max} in tribal patients suffering from malaria may result in slower clearance of parasites. Impaired chloroquine metabolism in the tribal population may have therapeutic relevance and needs to be investigated further to permit more appropriate and proper treatment of malaria patients in tribal populations of India.

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